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PROGRESS SUMMARY (Year 1) We have determined that the most phototactic wavelength is 1.7 μ m \pm 0.5 μ m.			
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ANNUAL PROGRESS REPORT ON CONTRACT N00014-89-J-1700

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Professor of Cell Biology
CONTRACTOR: Northwestern University Medical School, Chicago

CONTRACT TITLE: Cellular Detection of Infrared Sources

RESEARCH OBJECTIVE:

- a. Test for phototaxis of mammalian cells in culture in the infrared range of 1.2 - 5 μm wavelength.
- b. Test for the involvement of the centrosome in the phototactic response.

APPROACH: Using a specially designed microscope we observe 3T3 mouse fibroblasts in a live cell chamber through which a fine beam of infrared light passes. The wavelength of the beam is variable and its diameter (depending on wavelength) is 2 to 10 μm . Phototaxis is measured by the quantitation of the locomotion of the cells in the vicinity of the beam.

PROGRESS SUMMARY (Year 1) We have determined that the most phototactic wavelength is $1.7 \mu\text{m} \pm 0.5 \mu\text{m}$.

PROGRESS REPORT

BACKGROUND

Thesis 1: Animal cells are not merely chemical automata but possess data-processing and -integrating devices outside the nucleus that control their movement and perhaps other short-term cellular functions that relate to the environment.

Main technology: Phagokinetic tracks (Albrecht-Buehler, Cell 11:395 (1977)). They can be observed in some kind of biological cloud chamber in which cells migrate on a substrate covered with small gold particles and leave particle-free tracks behind.

Experimental support:

a. Existence of microplasts: It is possible to isolate small fragments, called 'microplasts' from live cells that express autonomously, but stereotypically the characteristic movements of whole cells. (Albrecht-Buehler, Proc. Natl. Acad. Sci. USA 77:6639 (1981)) *The existence of autonomously motile subdomains of whole cells demands a mechanism of body coordination of the cell, because the subdomains do not act independently in the intact cell.*

b. Symmetry and identity of sister cell tracks. Phagokinetic tracks of dividing 3T3 cells shows that the track of one sister is predominantly the mirror image or identical copy of the track of the other sister (Albrecht-Buehler, J. Cell Biol. 72:595 (1977)) *The results suggest the existence of programmed control of directional changes in cells.*

c. Collision figures of cells. Upon collision, 2 cells appear to invert 'vertical' component of their speed and retain the 'tangential' component, thus creating tracks that are reminiscent of elastic collisions between spherical objects. Such collisions, however, are certainly not brief elastic encounters but last up to 6 hours, nor are the cells spherical. *Therefore the collision figures suggest the existence of controlling actions that depend on complex assessments of the environment.*

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d. Probing actions of cells at intersections. Cells follow lines on a substrate independent of the chemical nature of the substrate as long as it is chemically inert. This phenomenon, called contact-guidance, can be used to observe cells at intersections between guiding lines (Albrecht-Buehler, J. Cell Biol. 80:53 (1979)). The combination of guiding substrates with the phagokinetic track technique shows that cells extend tentatively at intersections into all available directions. It can easily be shown that preferential attachment or differential retardation of the moving cells cannot play a role in the observation. It appears, therefore, that *the cellular control system is actively searching for input data from the environment.*

Thesis 2: The cytoplasmic control system is related to the pair of centrioles (centrosome) and uses microtubules as carriers of control signals.

Supporting facts and arguments:

a. *The front-rear polarity is microtubule dependent.* For many years it is known that migrating cells require intact microtubules to express 'polarity', i.e. a morphological distinction between a leading edge

b. *In animal cells the microtubules radiate unbranchingly from the centriolar area to the cell cortex that contains the autonomously motile microplasts.*

The centrioles are a pair of highly structured cylinders oriented at right angles to each other. Each cylinder consists of 9 blades that slant outward. Near the wall there are microtubular organizing centers from which the cytoplasmic microtubules radiate away. These microtubules cross the entire cytoplasm and terminate at the cell cortex from which we derived the autonomously motile microplasts. This 'anatomical' situation is consistent with the assumption, that the microtubules transmit 'control-signals' to the microplast domains.

c. *In migrating fibroblasts the pair of centrioles is oriented relative to the substrate and direction of migration.*

(See Albrecht-Buehler, Cell 12:333 (1977); Albrecht-Buehler and Bushnell, Exp. Cell Res. 120:111 (1979). This geometry suggests that the pair of centrioles plays a special role in the 'navigation' of the cells.

d. *The geometric features of the centriole pair are compatible with the design of a signal source detector.*

If cells were to 'see' objects in their vicinity, they could not use normal optical methods. Considering the typical cell size of $10\ \mu\text{m}$, a cellular 'eye-lense' could hardly be larger than $1\ \mu\text{m}$ and would have to focus radiation that is at least 1000 times smaller, i.e. in the x-ray range. Obviously, there are no focusing materials for x-rays, and in addition the radiation would be harmful to the cells. Yet, assuming that cells need to locate sources of radiation of much longer wavelength in the space around them, one can argue that the most appropriate method would require an instrument that would look exactly like a pair of centrioles (Albrecht-Buehler, Cell Motility 1:237 (1981)). The main arguments are summarized as follows. Assuming that the blades are able to absorb the radiation and that detectors for the particular radiation are located right behind the blades (Black dots in Fig. 1) there is one and only one receptor that can receive the radiation from the source S. In this way the (say) longitude of the source is mapped in a one-to-one fashion to a specific detector. In order to determine the latitude of the source the instrument would require a second identical cylinder at right angles, precisely as in the case of the centriole pair (Fig. 2). One can show that the principle allows continuous angular resolution and works for wavelengths which are considerably larger than the typical instrument dimensions.

PROGRESS

Thesis 3 (and objective of project): The wavelength that is detected by the pair of centrioles is in the infrared range.

Supporting arguments and progress:

a. *Suitability of infrared radiation.* Infrared is the predominant radiation around cells inside organisms (black body radiation at 300°). It carries characteristic information about the emitting structure. There are two major 'water windows' (1.2-3 μm and 3.5 to 6 μm) which would allow the radiation to penetrate the natural aqueous environment of cells.

b. *Construction of a suitable microscope and observation chamber.* The microscope that we developed sends a focused beam of infrared light to an inert object to scatter it. Cells surround the spot and are tested whether they migrate towards it. The infrared light originates in a remodelled spectrophotometer. In order to see the cells we had to develop a special phase contrast condenser with a hollow optical axis to allow the infrared light passage without absorption in any glass lenses. All materials that have to be passed by the infrared light are made of sapphire (sapphire cut-off = 7 μm). The specially developed chamber is only 100 μm thick in order to minimize water absorption.

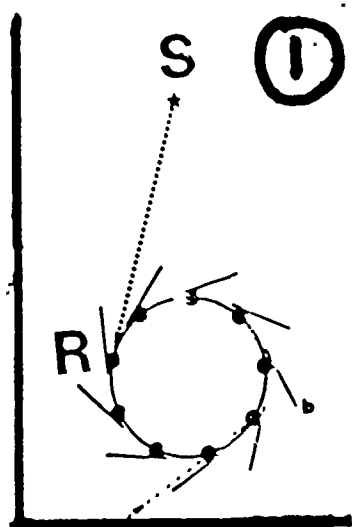
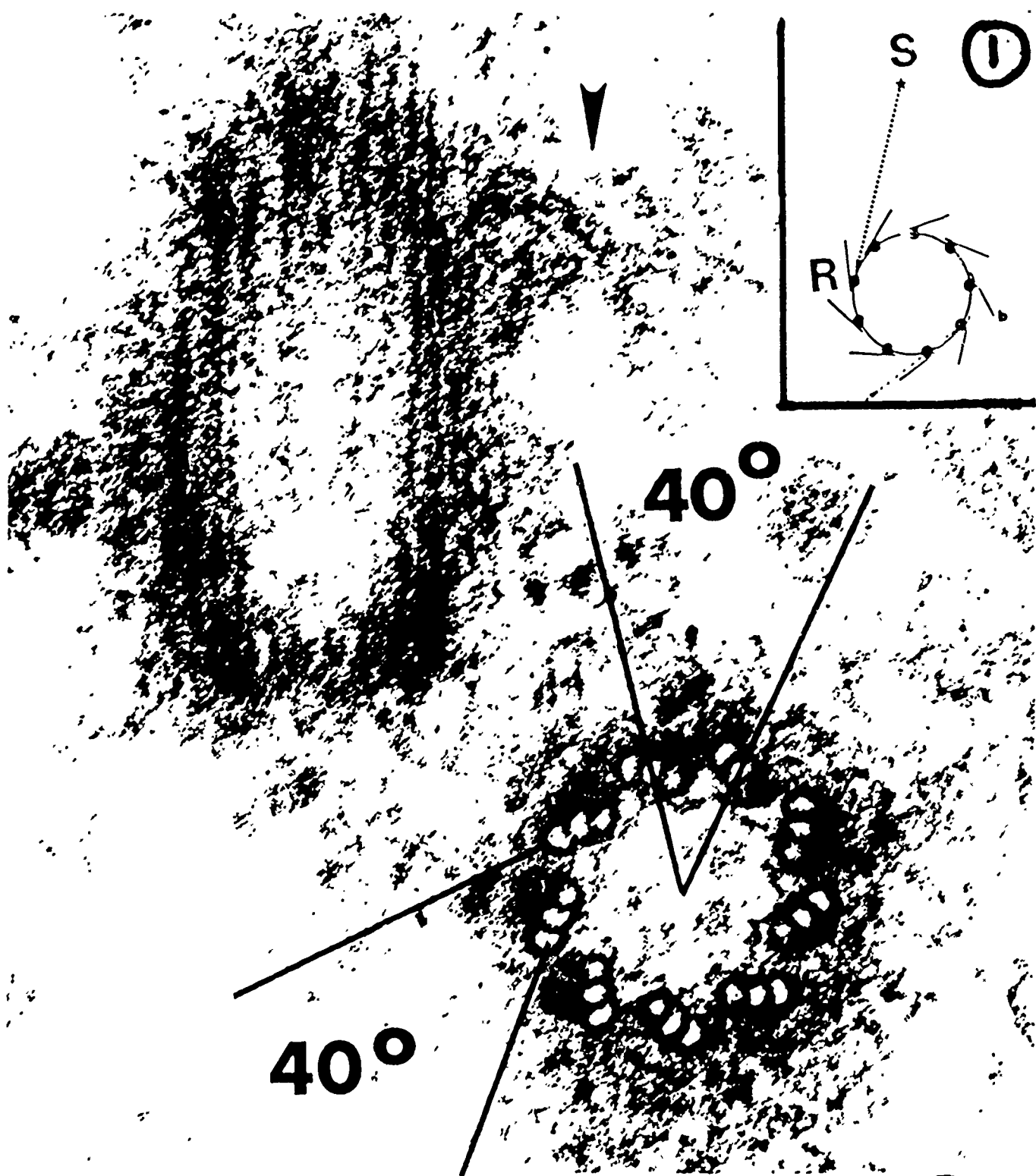
c. *The most phototactic wavelength appears to be $1.7 \pm 0.5 \mu\text{m}$.* We measured the locomotion of 3T3 mouse fibroblasts in the vicinity of the irradiated spot by video time lapse recording. As test wavelengths we used 1.2, 1.7, 2.2, 2.7, 3.2, 3.7, 4.2, and 4.7. For each wavelength we performed 10-15 independent experiments involving 6-12 individual cells each. The speed of locomotion was measured by the slope of the curve that measured the distance of each cell from the scattering spot as a function of time. Pooling all data we found that 67% of the cells moved towards the scattering spot. The strongest 'attraction' was found at a wavelength of 1.7 μm (average slope: -4 $\mu\text{m}/\text{h}$; range 1 to -8 $\mu\text{m}/\text{h}$), the weakest at 3.2 μm (average slope -1 $\mu\text{m}/\text{h}$; range 2 to -3 $\mu\text{m}/\text{h}$). Control experiments included the use of visible light (0.6 - 0.7 μm) and infrared light with a wavelength too long to penetrate the system (9 μm). In both cases we found only random migration of the cells with respect to the scattering spot.

d. *Tail extension experiments.* Based on these findings of the most phototactic infrared wavelength for cultured 3T3 mouse fibroblasts we have begun to verify the result with independent assays. If cells are able to direct their migration towards a source of infrared light of this wavelength, one should be able to use the wavelength to change the front-rear polarity of migrating cells directly.

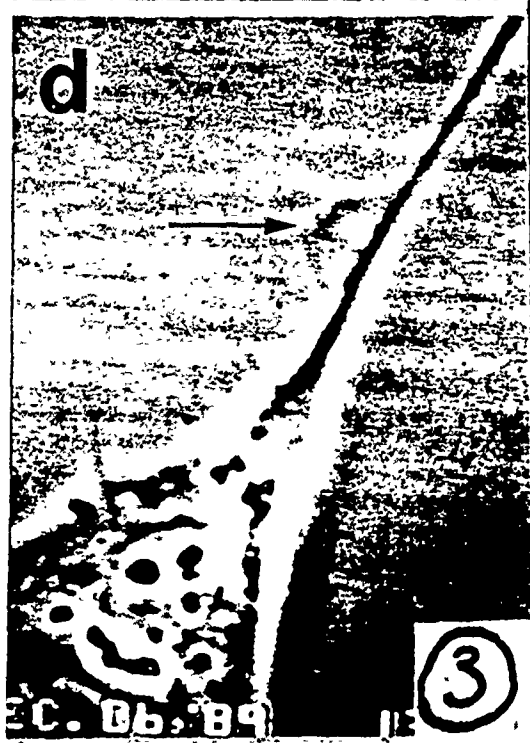
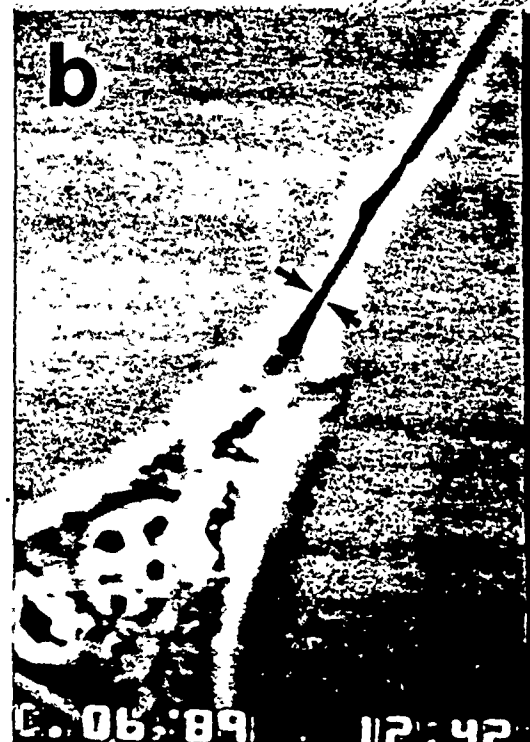
In order to test this prediction, we selected 56 migrating 3T3 cells with a well-formed tail (Fig. 3, panel a) and recorded their behavior for 30 min. During this time 66% of the cells moved their cytoplasm forward, as was demonstrated by the retraction or at least visible thinning of the tail (cf arrows in panels a and b). In 31% of the cases the tail thickness stayed constant or increased. In 3% we observed the rare phenomenon that a lamellipodium extended from the tail area as if the cell was about to reverse its front-rear polarity. Subsequently, we aimed for about 30 s at the root of the tail with a focussed spot of visible red light (circle in panel c) and then changed the wavelength to 1.7 μm (estimated power: 500 μW) for the following 30 min. During this time we found that only 43% of the cells continued to retract their tails. More impressively, 27% began to extend lamellipodia at the tail (see arrow in panel d). In 31% of the cases the tail thickness remained unchanged. The results suggest, that the illumination with the attractive infrared wavelength caused an 9-fold increase of the number of cells that appeared to initiate reversal of polarity.

The next experiments will include controls with other wavelengths, measurements of the intensity of the illumination and increasing the distance between the tail and the illuminating spot.

Preprints: Preprint of a paper about a new hypothesis of centriole formation is attached.



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